calculated from the bending of the MTF. Results showed that a significantly greater density of myotubes differentiated on laminin as compared to fibronectin, collagen IV, and collagen I lines. Next, we examined myotube formation on 20, 50, 100, and 200 μm line widths of LAM with line spacings of 10, 15, 20, and 30 μm and found that uniaxial myotube formation was achieved on widths less than 200 μm and spacings greater than 15 μm. MTFs grown on line widths ≥ 50 μm exhibited twitch stresses > 10 kPa. These results demonstrate that the skeletal MTF assay is a viable tool for elucidating how ECM composition and micropattering influence muscle formation and contractile function. Future work will focus on understanding how micropatterned ECM cues influence myogenesis and contractility of primary human myoblasts and establishing the MTF assay as a clinically relevant platform for *in vitro* drug screening.

Adipogenic Cell Sheets to Recreate *In Vitro* Adipose Tissue Microenvironments

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Cell sheet (CS) engineering, taking advantage of cellular self-matrix organized as in native tissue, has been largely explored, including by us, for different purposes [1–3]. Herein we propose for the first time, the use of human adipose stem cells (hASCs)-derived CS to create adipose tissue analogues with different levels of maturation. hASCs were cultured on UpCellTM thermo-responsive dishes for 1, 3 and 5 days under basal conditions previously established by us [3]. The influence of predifferentiation time and respective cell number, over CS stability and differentiation was assessed. Mechanically robust CS were only obtained with 5 days pre-differentiation period. Adipogenesis was followed along the culture assessing the variation of expression of mesenchymal (CD73, CD105 but not CD90) and adipogenic (PPARγ, FABP4 and LPL) markers by flow cytometry, immunocytochemistry and RT-PCR. Increased ratio of differentiated cells was achieved for longer predifferentiation periods, while maturation degree was modulated by the maintenance medium. Independently of the overall CS differentiation/maturation level, 3D constructs were fabricated by stacking and further culturing 3 CS. Thus, by varying the culture conditions, different 3D adipose tissue-like microenvironments were recreated, enabling future development of new tissue engineering strategies, as well as further study of adipose tissue role in the regeneration of different tissues.

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An Irradiation-Injection Approach to Study Tendon Stem Cell Differentiation in a Mouse Model

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Tendinopathy is a prevalent tendon disorder that affects millions of Americans and costs billions of healthcare dollars every year. Current clinical treatments for tendinopathy are largely palliative because the precise cellular and molecular mechanisms of the disorder are not defined. Previously, using an *in vitro* model we showed that the leading cause of tendinopathy is the aberrant differentiation of tendon stem cells (TSCs) into non-tenocytes when subjected to mechanical over-loading [1]. Under identical conditions, the resident tendon cells or tenocytes did not differentiate into non-tenocytes *in vitro* [2]. However, it is not clear whether tendon cells (TSCs and tenocytes) *in vivo* also behave in a similar fashion. Therefore, in this study we used a novel irradiation-and-injection approach to determine whether native TSCs could be eliminated or reduced by irradiation thus enabling tracking of GFP tagged TSCs in the injected region. Histology results showed that these injected GFP-TSCs differentiated into no-tenocytes after intensive treadmill running. This approach can reveal the precise fate of TSCs *in vivo* and can offer more insights to the mechanisms causing tendinopathy. Our results indicate that the irradiation and injection approach has the following advantages: It can be used successfully to obtain pure TSCs *in vivo* without potential cell contamination from tenocytes. It is an effective approach to determine the role of TSCs in tendon homeostasis and tendon pathology or tendinopathy. This approach is feasible and can be routinely used in laboratories to perform basic science research.

Fibroblast Behavior in an Injectable Covalently Cross-Linkable Gelatin Matrix Incorporating Select Growth Factors

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An injectable matrix capable of replacing lost stroma, and stimulating the proliferation and migration of endogenous fibroblastic cells, could enable the early treatment of an array of musculoskeletal defects, including those in the annulus fibrosus of intervertebral disk. Gelatin, which has adhesion ligands for fibroblasts and endothelial cells and the ability to undergo enzymatic degradation, was conjugated with hydroxyphenyl propionic acid (Gtn-HPA) in order to enable independent tuning of the gelation time and degree of covalent cross-linking *in vivo* by horseradish peroxidase and peroxide (1). NIH 3T3 fibroblasts seeded in Gtn-HPA and exposed to the cross-linking process survived with 95 ± 1% viability. The proliferative and migration behavior of the cells was compared when the following growth factors were incorporated into the Gtn-HPA: TGF-β1, FGF-2, PDGF-BB, EGF. On day 7, Gtn-HPA incorporating TGF-β1 or FGF-2 increased the cell numbers for ~2 folds compared to controls, in a proliferation assay. A migration assay, designed as ''annulus-core'' system in which cells migrate from an annular cell-seeded type I collagen gel into growth factor-loaded Gtn-HPA cores, demonstrated increases in the cell number, clustering, and depth of migration into the Gtn-HPA when TGF-β1 was incorporated into the Gtn-HPA. The results demonstrate the promise of this injectable gelatin-based matrix for treatment of select early-stage musculoskeletal defects/ruptures.

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Evaluation of the Immunomodulatory Potential of a Chitosan-graft-poly(ε-Caprolactone) Copolymer

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Recently we synthesized a copolymer consisting of poly(ε-caprolactone) chains chemically grafted on a chitosan backbone (CS-g-PCL) and we showed that it can support the growth of Wharton's jelly Mesenchymal Stem Cells providing a material with potential in cardiovascular applications [1]. An essential part of the biocompatibility assessment includes the investigation of the immune response against the biomaterial indented for *in situ* regeneration. The aim of the current study is to evaluate the potential of CS-g-PCL to modulate different functions of innate and adaptive immune response under various stimuli. Specifically, we study the effect of CS-g-PCL on the profile of secreted cytokines in spleen cultures and on the proliferation of T and B lymphocytes. Additionally, we analyzed the influence of the biomaterial in the polarization of bone marrow-derived macrophages (BMDM), since the transition from M1 to M2 phenotype is beneficial for tissue remodeling upon biomaterial implantation. The results show that although CS-g-PCL does not induce an immune response, it induces specific effects on immune cells. CS-g-PCL shows anti-inflammatory action in BMDM culture by reducing the